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Prevalence of Novel Candidate Sjogren Syndrome Autoantibodies in the Dry Eye Assessment and Management (DREAM) Study

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Purpose: To evaluate the prevalence of novel candidate Sjogren syndrome (SS) autoantibodies [salivary protein-1 (SP-1), parotid secretory protein, carbonic anhydrase 6] in the DRy Eye Assessment and Management (DREAM) cohort, a study evaluating the effectiveness of omega-3 fatty acid supplements for the treatment of dry eye.

Methods: Participants underwent ocular surface examinations and serological testing for traditional and novel SS autoantibodies. Dry eye assessment and management participants were categorized into the following 3 groups: 1) no history of SS or other autoimmune diseases and negative traditional SS autoantibodies (n = 352); 2) no history of SS but a history of other autoimmune diseases (n = 66); and 3) those who met the 2012 American College of Rheumatology SS classification criteria (n = 52).

Results: Eleven percent had a history of SS, and 6% of those without a history of SS most likely had undiagnosed SS. The SS group had a higher prevalence of SP-1 autoantibodies than the group without SS or other autoimmune diseases (33% vs. 19%; $P = 0.02$) but had no difference in carbonic anhydrase 6 ($P = 0.31$) or parotid secretory protein autoantibodies ($P = 0.33$). Participants who were positive for the traditional autoantibodies alone or positive for both traditional and novel autoantibodies had the highest scores for corneal ($P = 0.002$) and conjunctival staining ($P < 0.001$).

Conclusions: Data from this multicenter, prospective study demonstrated that one of the novel candidate autoantibodies, SP-1, is associated with underlying SS and that novel autoantibodies may be associated with worse ocular surface disease. Future longitudinal studies are needed to evaluate their utility in screening patients with dry eye for SS.

Key Words: Sjogren syndrome, novel antibodies, dry eye

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Sjogren syndrome (SS) is one of the most common autoimmune diseases, affecting 2 to 4 million people in the United States alone.[1,2] SS is characterized by lymphocytic infiltration of the exocrine glands, for example, lacrimal glands and salivary glands, leading to symptoms of dry eye and dry mouth. Patients with SS also have increased autoantibody production and a higher risk of lymphoma.[3] Signs and symptoms of SS span the domains of ophthalmology, endocrinology, and rheumatology, and the diagnostic criteria are complex.[4–11] The diversity of signs and symptoms is a barrier to early diagnosis, and many patients with SS are undiagnosed.[4,12,13] Early detection of SS is important so that treatments can be implemented to relieve symptoms and to enable monitoring for systemic complications. In addition, patients

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who are administered biological agent treatment within the first 5 years of disease onset may be more likely to respond to treatment than those with delayed initiation of therapy.[14–16]

More specific and sensitive markers for SS are needed to allow for earlier diagnosis and timely management of patients. The traditional autoantibodies to SS-related antigen A (SSA/Ro) and SS-related antigen B (SSB/La)[1,17] are present in only 50% to 70% of patients with SS.[17,18] In addition, because traditional SS autoantibodies appear late in the course of disease,[19] patients with early SS are often negative for these antibodies, thereby contributing to delays in diagnosis.

Recently, the novel autoantibodies salivary protein 1 (SP-1), carbonic anhydrase 6 (CA-6), and parotid secretory protein (PSP) have been identified as early markers of disease in a mouse model of SS.[20] However, there are limited data regarding the expression and clinical significance of these antibodies in humans. These novel markers have been shown to be present in some patients with dry eye with or without SS in a few small studies[21−25], but studies examining the prevalence of these antibodies in large, well-characterized cohorts are needed to understand the clinical significance of these autoantibodies. In addition, information on how the expression of these autoantibodies changes over time is needed.

The Dry Eye Assessment and Management (DREAM) Study is a multicenter clinical trial funded by the National Eye Institute, National Institutes of Health, to examine the efficacy and safety of an oral omega-3 fatty acid supplement for the treatment of dry eye. Both dry eye patients with SS and those without SS were enrolled in DREAM. The data from the DREAM Study present a unique opportunity to assess the prevalence of these novel candidate SS autoantibodies and any associated ocular surface phenotypic features in a well-characterized cohort of SS and non-SS dry eye patients.

METHODS

Subjects

The DREAM Study was a prospective, randomized, double-masked, superiority clinical trial (clinicaltrials.gov: NCT02128763) involving an active supplement group and a placebo group. Participants were enrolled from 27 centers in 17 states throughout the United States. Institutional Review Board/Ethics Committee approval was obtained. In addition, the study adhered to the tenets of the Declaration of Helsinki and was HIPAA compliant. After written informed consent was obtained, participants who had at least 1 eye meeting the DREAM criteria for dry eye were enrolled. Inclusion criteria were age greater than or equal to 18 years; dry eye-related ocular symptoms for at least 6 months before the screening visit; and the use or desire to use artificial tears on average 2 times per day in the 2 weeks before the screening visit. Participants also had to demonstrate the presence of at least 2 of the 4 following signs in the same eye at the screening visit and eligibility confirmation visits: 1) conjunctival staining score ≥1 (out of a possible score of 6 per eye); 2) corneal fluorescein staining present ≥4 (out of a possible score of 15 per eye); 3) tear breakup time ≤7 seconds; and 4) anesthetized Schirmer test score ≥1 to ≤7 mm/5 min. In addition, participants had to report symptoms of dry eye with an Ocular Surface Disease Index (OSDI) score of at least 25 (≥25 to ≤80) at the screening visit and at least 21 (≥21 to ≤80) at the baseline randomization visit. Finally, participants had to demonstrate compliance with taking placebo softgels as directed during a 2-week run-in period (≥90% taken, by pill count).

Major exclusion criteria were the following: the presence of acute allergic conjunctivitis, infection, or inflammation; history of ocular herpes keratitis; ocular surgery within 6 months; history of previous LASIK or other corneal surgery; use of glaucoma medication or history of filtering surgery for glaucoma; eyelid abnormalities or extensive ocular surface scarring; anticoagulation therapy; contact lens wear within 30 days of screening visit; current use of eicosapentanoic acid/ docosahexanoic acid (EPA/DHA) supplements greater than 1200 mg/day; and a history of allergy to ingredients of supplements (active or placebo).

During the eligibility confirmation visit, clinical coordinators asked patients about their medical history, including specific items on SS and rheumatoid arthritis. Participants provided information regarding diagnoses of other autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosus, fibromyalgia, calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia), antiphospholipid syndrome, Raynaud disease, scleroderma, graft-versus-host disease, and sarcoidosis) when answering review of systems questions. Patients provided a 4- to 10-mL blood sample that was sent to a central laboratory for masked analysis of traditional and novel SS autoantibodies (Sjo test; Immco Diagnostics, Inc, Buffalo, NY). A standard enzyme-linked immunosorbent assay for antibodies to SS antigens was used to detect immunoglobulin G, immunoglobulin A, and immunoglobulin M antibodies in the human serum extract reactive to recombinant SP-1, CA-6, and PSP proteins expressed and purified from *Escherichia coli*. Results were expressed in enzyme-linked immunosorbent assay units per milliliter and were reported as positive or negative as defined by the manufacturer.[25] Results of testing were made available to the patient and the treating physician after they exited the DREAM Study.

Designation of Sjogren Syndrome Status

We used the 2012 American College of Rheumatology (ACR) criteria for SS[10] as the basis for classifying DREAM patients. The ACR criteria require that at least 2 of the following 3 criteria be met: 1) positive for the traditional SS antibodies [positive for SSA or positive for SSB or (positive for rheumatoid factor and ANA ≥1:320)]; 2) ocular staining score (OSS) from the cornea and conjunctiva of 3 or more in the worse eye; and 3) labial salivary gland biopsy exhibiting focal lymphocytic sialadenitis with a focus score of 1 focus/4 mm^2. Labial salivary gland biopsy results were not available for DREAM patients. The OSS was not used in the DREAM study; however, for each eye, the corneal fluorescein staining score (NEI scale; scores 0–15) was added to the conjunctival lissamine green staining score (modified Oxford scale; scores 0–6). We estimated that a total sum of corneal and conjunctival staining of 3 or more was equivalent to an OSS score of 3 or more. DREAM patients were classified as

—1) group 1 (control group): those with an autoantibody profile that did not fulfill ACR criteria and without a reported history of SS or other autoimmune diseases; 2) group 2: those with an antibody profile that did not meet ACR criteria, without a reported history of SS but with a history of other autoimmune diseases; and 3) group 3: those with an antibody profile that met ACR criteria and with a score of ≥3 on DREAM ocular surface staining tests (SS group).

Data Analysis

The primary analysis compared the SS group (group 3) and the control group (group 1) on the baseline characteristics and prevalence of each of the novel autoantibodies using the 2-sample t test for means and the Fisher exact test for proportions. Secondary analyses compared the autoimmune disease group (group 2) and the control group for their baseline characteristics and prevalence of antibodies.

To evaluate whether SS antibodies were associated with more severe dry eye disease, signs and symptoms of dry eye were compared among the following 4 groups of participants based on their traditional and novel autoantibody status: 1) positive for only the traditional autoantibodies; 2) positive for only the novel autoantibodies; 3) positive for both traditional and novel autoantibodies; and 4) negative for both traditional and novel autoantibodies. All statistical analyses were performed in SAS v9.4 (SAS Institute Inc, Cary, NC), and $P < 0.05$ was considered statistically significant.

RESULTS

Among 535 patients randomized to the DREAM study, 494 underwent antibody testing (Fig. 1). Antibody testing was not performed when a licensed phlebotomist was unavailable during the patient visit, the patient refused, or the appropriate shipping materials were not available. Among those with antibody testing, 52 (10.5%) patients met the ACR criteria for inclusion in group 3 with SS, 66 (13.4%) reported an autoimmune disease to qualify for group 2, and 352 (71.3%) reported no history of SS or autoimmune disease and were included in the control group (group 1). Twenty-four patients (4.9%) either reported a history of SS or had an antibody profile meeting ACR SS criteria but did not meet the full ACR criteria and were considered indeterminate.

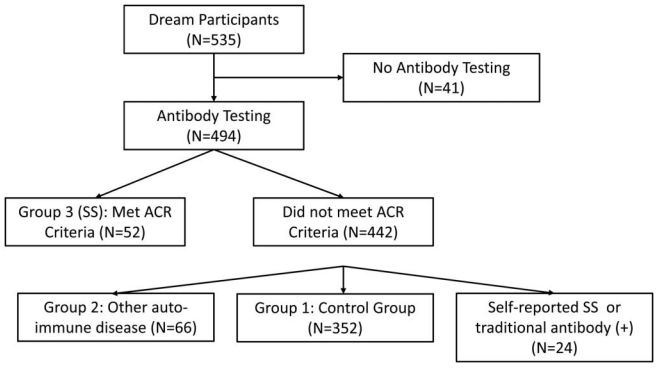

FIGURE 1. Flowchart of the analysis of DREAM study participants regarding SS and autoantibody testing.

Baseline Characteristics

The baseline characteristics of the participants with SS (group 3) compared with those without SS or other autoimmune diseases (group 1) are shown in Table 1. Participants with SS were predominantly female (92%) and predominantly white. There was no significant difference in the mean OSDI score between these 2 groups. However, the 4 key signs of dry eye, tear breakup, Schirmer test, and corneal and conjunctival staining, were significantly worse in participants with SS than in those without SS or other autoimmune diseases (all $P \leq 0.02$). Also, participants with SS used artificial tears ($P = 0.004$) or ointments ($P = 0.01$) more frequently than did those without SS or those with other autoimmune diseases.

TABLE 1. Baseline Characteristics of DREAM Study Participants With or Without SS

Baseline Characteristics	Group 1: No SS and No Other Autoimmune Diseases* (n = 352)	Group 3: SS (N = 52)†	P
Age (yr): mean (SD)	58.8 (13.6)	56.6 (12.5)	0.91
Sex: male (%)	76 (22)	4 (8)	0.02
Race, n (%)			0.63
White	274 (78)	42 (81)	
African American	39 (11)	4 (8)	
Asian	13 (4)	1 (2)	
American Indian or Alaskan native	2 (1)	0 (0)	
Mixed	4 (1)	2 (4)	
Unknown	20 (6)	3 (6)	
OSDI score: mean (SD)	41.4 (15.1)	42.8 (15.6)	0.55
Tear break-up time (s): mean (SD) ‡	2.7 (1.4)	2.2 (1.1)	0.001
Schirmer test score (mm): mean (SD)‡	8.4 (6.3)	6.3 (5.3)	0.02
Fluorescein staining of the cornea: mean (SD) ‡	4.2 (2.9)	6.0 (3.6)	0.001
Lissamine green staining of conjunctiva: mean (SD)‡	3.2 (1.4)	4.4 (1.5)	<0.0001
Frequency of using artificial tears or gel use in the last week, n (%)			0.004
0	80 (23)	5 (10)	
1–2 times	160 (45)	19 (37)	
3–4 times	65 (19)	11 (21)	
5–10 times	32 (9)	11 (21)	
Greater than 10 times	15 (4)	6 (12)	
Regularly use lubricating ointment (%)	29 (8)	10 (19)	0.01
Ever used steroid eye drops or ointment (%)	74 (21)	17 (33)	0.06

*Group 1: participants without a history of SS or other autoimmune diseases and negative in SSA, SSB, and not positive for both RF and ANA.

†Group 3: participants who would likely have met the 2012 American College of Rheumatology SS classification criteria based on serology and ocular surface staining.

‡From the worse eye of a specific ocular dry eye measurement.

Novel Candidate SS Antibodies

Participants with SS had a higher prevalence (46%) of expressing at least 1 novel autoantibody compared with those without SS or other autoimmune diseases (31%; $P = 0.02$) (Table 2). In particular, participants with SS had a higher prevalence (33%) of SP-1 autoantibodies than those without SS or other autoimmune diseases (19%; $P = 0.02$). However, there was no significant difference in the prevalence of CA-6 autoantibodies (21% vs. 15%; $P = 0.31$) or in PSP autoantibodies (9.4% vs. 13.5%; $P = 0.33$).

Comparison by Antibody Groups

Among 4 groups based on testing results of the traditional and novel candidate SS autoantibodies, demographic and ocular characteristics were similar (Table 3). Participants who were positive for the traditional autoantibodies alone or positive for both traditional and novel autoantibodies had the highest scores for corneal staining ($P = 0.002$) and conjunctival staining ($P < 0.001$) (Table 3).

Comparison of Non-SS Patients With or Without Other Autoimmune Diseases

In a secondary analysis, the baseline characteristics and the autoantibody status of 66 participants without SS but with a history of another autoimmune disease (group 2) were examined and compared with those without SS or other autoimmune diseases (group 1) (see Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/ICO/A700, and Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/ICO/A701). Approximately half (53%) of the participants with another autoimmune disease (group 2) reported having ongoing rheumatoid arthritis. Participants in both of these groups (groups 1 and 2) were of similar age and sex. There was a higher proportion of African American patients in group 2 than in group 1 (21% vs. 11%; $P = 0.04$). The mean OSDI score was similar between groups ($P = 0.39$). The mean score for each of the signs of dry eye was worse in group 2 (those with other autoimmune diseases) than in group 1 (no SS or other autoimmune diseases), but none of the differences was statistically significant (all $P \geq 0.08$) (see Supplemental Table 1, Supplemental Digital Content 1, http://links.lww.com/ICO/A700). There was a significantly higher prevalence of autoantibodies to CA-6 (25% vs. 15%; $P = 0.047$) and PSP (18% vs. 9%; $P = 0.049$) in group 1 compared with group 2. However, there was no difference in the prevalence of anti-SP-1 antibodies (group 1: 19% vs. group 2: 23%; $P = 0.4$) (see Supplemental Table 2, Supplemental Digital Content 2, http://links.lww.com/ICO/A701).

Correlation Between Traditional and Novel SS Antibodies

Among all participants (groups 1–3) who underwent testing for each autoantibody (n = 492), there was a weak correlation (Pearson correlation coefficient = 0.17; $P = 0.0002$) between the number of participants who were traditional autoantibody positive and the number who were novel autoantibody positive (see Supplemental Table 3, Supplemental Digital Content 3, http://links.lww.com/ICO/A702).

TABLE 2. Antibody Testing Results of DREAM Study Participants With or Without SS

Baseline Antibodies	Group 1: No SS and No Other Autoimmune Diseases* (n = 352)	Group 3: SS† (N = 52)	Fisher Exact P‡
Traditional SS antibodies:			
SS-A (Ro) >25 EU/mL	0 (0.0%)	48 (92.3%)	
SS-B (La) >25 EU/mL	0 (0.0%)	15 (28.9%)	
Positive tests for SS-A (Ro) and SS-B(La)			
0	352 (100%)	2 (3.9%)	
1	0 (0.0%)	37 (71.2%)	
2	0 (0.0%)	13 (25.0%)	
Anti-nuclear antibody ≥1:320§	13 (3.7%)	23 (44.2%)	
Rheumatoid factor: any positive§	81 (23.1%)	32 (61.5%)	
Positive tests for traditional antibodies			
0	258 (73.3%)	0 (0.0%)	
1	94 (26.7%)	14 (26.9%)	
2	0 (0.0%)	20 (38.5%)	
3	0 (0.0%)	8 (15.4%)	
4	0 (0.0%)	10 (19.2%)	
Novel SS antibodies§			
Salivary protein 1 (SP-1): any positive	65 (18.5%)	17 (32.7%)	0.02
Carbonic anhydrase VI (CA VI): any positive	53 (15.1%)	11 (21.2%)	0.31
Parotid specific protein (PSP): any positive	33 (9.4%)	7 (13.5%)	0.33
Positive in any novel antibody	107 (30.5%)	24 (46.2%)	0.02
Positive tests for novel antibodies			0.03‖
0	244 (69.3%)	28 (53.9%)	
1	66 (18.8%)	16 (30.8%)	
2	38 (10.8%)	5 (9.6%)	
3	3 (0.9%)	3 (5.8%)	

*Group 1: participants without a history of SS or other autoimmune diseases and negative in SSA, SSB, and not positive for both RF and ANA.

†Group 3: participants who would likely have met the 2012 American College of Rheumatology SS classification criteria based on serology and ocular surface staining.

‡No *P* values are provided for traditional SS antibodies because these values were used to define the 2 comparison groups.

§One patient in the non-SS group had missing data for antibody testing.

‖For test of a linear trend.

EU, enzyme-linked immunosorbent assay units.

DISCUSSION

In this study, we found that participants with SS had a significantly higher prevalence of SP-1 autoantibodies compared with those without SS or other autoimmune diseases. However, the prevalence of the novel autoantibodies in both SS

TABLE 3. Comparison of Demographics, Dry Eye Symptoms, Signs, and Treatment Among SS Autoantibody Groups of Participants in the DREAM Study*

Demographics	Negative for Traditional and Novel SS Antibodies (n = 214)	Positive for Only Novel SS Antibodies (n = 85)	Positive for Only Traditional SS Antibodies (n = 86)	Positive for Traditional and Novel SS Antibodies (n = 63)	*P*
Age (yr); mean (SD)	58.8 (13.3)	59.0 (12.7)	58.6 (12.7)	59.5 (15.3)	0.98
Sex: male (%)	45 (21.0)	16 (18.8)	17 (19.8)	8 (12.7)	0.53
Race, n (%)					0.03
White	169 (79.0)	67 (78.8)	54 (62.8)	53 (84.1)	
African American	19 (8.9)	13 (15.3)	13 (15.1)	9 (14.3)	
Asian	7 (3.3)	1 (1.2)	8 (9.3)	0 (0.0)	
American Indian or Alaskan native	2 (0.9)	0 (0.0)	1 (1.2)	0 (0.0)	
Mixed	3 (1.4)	2 (2.4)	2 (2.3)	1 (1.6)	
Unknown	14 (6.5)	2 (2.4)	8 (9.3)	3 (3.9)	
OSDI score: mean (SD)	42.1 (15.0)	42.7 (16.6)	38.8 (14.6)	42.6 (15.3)	0.3
Tear breakup time (s): mean (SD)†	2.8 (1.4)	2.7 (1.4)	2.6 (1.4)	2.3 (0.8)	0.09
Schirmer test score (mm): mean (SD)†	8.5 (6.5)	8.0 (6.0)	7.4 (5.7)	7.6 (6.3)	0.49
Staining of the cornea: mean (SD)†	4.1 (2.9)	4.3 (2.8)	4.9 (3.2)	5.7 (3.6)	0.002
Staining of conjunctiva: mean (SD)†	3.0 (1.4)	3.1 (1.5)	4.0 (1.5)	3.7 (1.6)	<0.001
Frequency of using artificial tears or gel use in the last week, n (%)					0.7
0	49 (22.9)	18 (21.2)	20 (23.3)	11 (17.5)	
1–2 times	83 (38.8)	43 (50.6)	34 (39.5)	30 (47.6)	
3–4 times	47 (22.0)	12 (14.1)	20 (23.3)	14 (22.2)	
5–10 times	23 (10.8)	10 (11.8)	6 (7.0)	5 (7.9)	
Greater than 10 times	12 (5.6)	2 (2.4)	7 (7.0)	3 (4.8)	
Regularly use lubricating ointment, n (%)	16 (7.5)	14 (16.5)	12 (14.0)	7 (11.1)	0.11
Ever used steroid eye drops or ointment, n (%)	36 (16.8)	23 (27.1)	23 (26.7)	19 (30.2)	0.047

*One participant without novel antibody results was excluded.
†From the worse eye of a specific ocular dry eye measurement.

and non-SS participants in our study differed from that of previous reports.[20–22,24–26] There are a few possible explanations for the difference between the prevalence rates of the novel autoantibodies in our study and those of previously published studies. Factors such as differences in the classification criteria used to define SS, duration of disease, age, sex, and/or race and ethnicity could account for the varying prevalence rates seen across studies.

We also found that participants who were positive for the traditional SS autoantibodies alone, or for both traditional and novel autoantibodies, had worse corneal and conjunctival staining than those who were not positive for any of these autoantibodies. These novel autoantibodies may be a marker of more severe ocular surface disease in those positive for traditional SS antibodies. Future longitudinal studies are needed to examine the time course for both traditional and novel SS autoantibodies and to determine whether or not ocular surface damage progresses more quickly among those with specific subtypes of autoantibodies.

This study has certain limitations. First, the assignment of case status of SS was based on a combination of traditional SS antibody status and the ocular surface examination. However, we did not have information on previous SS workups, and some participants had never undergone lip biopsies. This could have resulted in some misclassification bias in that some patients in the non-SS dry eye group may have had undiagnosed SS, whereas some in the SS group may not have truly had SS. This potential misclassification would have diluted any real difference in the prevalence of autoantibodies between both groups. However, we found that the prevalence of traditional SS autoantibodies in our participants with SS was similar to the prevalence reported in well-characterized groups of patients with SS, which supports our classification of SS and non-SS groups.[20,27] In addition, we defined our SS group as only those who would have met the 2012 ACR classification criteria for SS.

An additional limitation is that our SS group was comprised of 52 participants. Therefore, our finding that there was no significant difference in novel autoantibody prevalence between groups could be the result of a low statistical power to detect a difference or a lack of true association. Future larger studies would be helpful in confirming our results. Another limitation is that we did not have information about the duration of SS. Because the novel candidate SS autoantibodies were detected early in the course of disease in a mouse model, these antibodies may be more likely to be present in patients with early SS.[20] It is possible that if many SS patients in our cohort had longstanding disease then this would result in a lower proportion of them to expressing these novel autoantibodies. Longitudinal studies that assess the impact of the duration of SS are needed. Finally, our cohort did not include any participants

without dry eye. Future studies that include this subgroup would be helpful in comparing the prevalence of the novel autoantibodies in those with or without dry eye.

The traditional SS autoantibodies have limitations in specificity and sensitivity; therefore, there is a need for better biomarkers for SS. For example, recently, it was shown that SSB antibodies, in the absence of SSA antibodies, were not associated with key phenotypic features of SS,[28] and as a result, anti-SSB is not included in the latest set of classification criteria for SS.[11] We found a weak correlation between traditional SS antibody-positive results and novel candidate SS-antibody positive results. Although the novel candidate SS autoantibodies have shown promise in a mouse model for SS, their meaning and clinical utility in humans need to be studied further, including the sensitivity and specificity of these antibodies for the early diagnosis of SS in humans. In addition, the meaning of positivity of the different isotypes of each novel autoantibody is unknown. Finally, it is important to remember that these novel candidate SS autoantibodies are not currently part of any of the classification criteria sets for SS.[9–11]

We also found that approximately 11% of our dry eye patient cohort reported having a history of SS, which is consistent with previous reports.[27] However, 6% of dry eye patients without a history of SS most likely had undiagnosed SS based on the 2012 ACR criteria, underscoring the need for improved screening methods and referrals for timely systemic evaluations for SS.

In conclusion, the DREAM clinical trial provides the largest data set to date that allows for the examination of the prevalence of novel candidate SS autoantibodies in dry eye patients with or without SS. We found that dry eye patients with SS had a significantly higher prevalence of SP-1 autoantibodies compared with those without SS or other autoimmune diseases. In addition, among those with traditional SS autoantibodies, the concomitant presence of the novel autoantibodies may be a marker of more severe ocular surface disease. Longitudinal data regarding autoantibody expression over time will be useful in further examining the patterns of expression in SS and non-SS dry eye patients and correlations with clinical disease.

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